Research, Society and Development, v. 11, n. 9, e37211931988, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i9.31988

Impact of different marketing conditions on the bacteriological quality of meat products

Impacto das diferentes condições de comercialização na qualidade bacteriológica de produtos cárneos

Impacto de las diferentes condiciones de comercialización en la calidad bacteriológica de los productos cárnicos

Received: 06/20/2022 | Reviewed: 07/01/2022 | Accept: 07/03/2022 | Published: 07/13/2022

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Abstract
Animal foods, such as meat and meat products, are among the main sources of infection and spread of Foodborne Diseases, where marketing conditions are an important factor to be evaluated. Forty-eight samples of meat products sold under outdoor fairs, butcher shops and supermarkets were collected. The presence of microorganisms indicating hygienic and sanitary quality (fecal coliforms and *Clostridium perfringens*) was investigated, as well as the species *Pseudomonas aeruginosa*. The results showed a high presence of *C. perfringens* in 83.33% of the samples, fecal coliforms in 73% and *P. aeruginosa* in 100% of meat products, in all commercial points. As for marketing conditions, the difference in the food contamination index was significant ($p \leq 0.05$) only for *C. perfringens* ($X^2_{(2)} = 8.5$; $p = 0.0143$), where meat sold in fairs were more susceptible to contamination by *C. perfringens* than meats sold in supermarkets. The meat products analyzed did not show quality from a bacteriological point of view, given the high presence of fecal coliforms, *P. aeruginosa* and *C. perfringens*, leading to question mainly the form of trade that can generate unsafe products for consumption.
Keywords: Foodborne diseases; Meat products; Food safety.

Resumo

Alimentos de origem animal, como carnes e derivados, estão entre as principais fontes de infecção e disseminação de Doenças Transmitidas por Alimentos, onde as condições de comércio são um importante fator a ser avaliado. Foram coletadas 48 amostras de produtos cárneos comercializados em feiras, açougues e supermercados. Foram pesquisados microrganismos indicadores da qualidade higiênica e sanitária (coliformes fecais e *Clostridium perfringens*) e pesquisa da espécie *Pseudomonas aeruginosa*. Os resultados revelaram alta presença de *C. perfringens* em 83,33% das amostras, coliformes fecais em 73% e *P. aeruginosa* em 100% dos produtos cárneos, em todos os pontos comerciais analisados. Quanto às condições de comercialização, a diferença no índice de contaminação de alimentos foi significativa ($p \leq 0,05$) apenas para *C. perfringens* ($X^2_{(2)} = 8,5$; $p = 0,0143$), onde as carnes vendidas em feiras foram mais susceptíveis à contaminação por *C. perfringens* do que as carnes vendidas em supermercados. Os produtos cárneos analisados não apresentaram qualidade do ponto vista bacteriológico, haja vista a elevada presença de coliformes fecais, *P. aeruginosa* e *C. perfringens*, levando-se a questionar principalmente a forma de comércio que pode gerar produtos inseguros para o consumo.

Palavras-chave: Doenças transmitidas por alimentos; Produtos cárneos; Inocuidade dos alimentos.

Resumen

Los alimentos de origen animal, como la carne y los productos cárnicos, se encuentran entre las principales fuentes de infección y propagación de enfermedades transmitidas por los alimentos, donde las condiciones comerciales son un factor importante a evaluar. Se recolectaron 48 muestras de productos cárnicos vendidos en ferias, carnicerías y supermercados. Se investigaron microorganismos indicadores de calidad higiénica y sanitaria (coliformes fecales y *Clostridium perfringens*) y la especie *Pseudomonas aeruginosa*. Los resultados revelaron una alta presencia de C. perfringens en el 83,33% de las muestras, coliformes fecales en el 73% y *P. aeruginosa* en el 100% de los productos cárnicos, en todos los puntos comerciales analizados. En cuanto a las condiciones de comercialización, la diferencia en el índice de contaminación de alimentos fue significativa ($p \leq 0,05$) solo para *C. perfringens* ($X^2_{(2)} = 8,5$; $p = 0,0143$), donde las carnes vendidas en ferias fueron más susceptibles a la contaminación por C. perfringens que la carne que se vende en los supermercados. Los productos cárnicos analizados no mostraron calidad desde el punto de vista bacteriológico, dada la alta presencia de coliformes fecales, *P. aeruginosa* y *C. perfringens*, lo que llevó a cuestionar principalmente la forma de comercio que puede generar productos inseguros para el consumo.

Palabras clave: Enfermedades transmitidas por alimentos; Productos cárnicos; Inocuidad de los alimentos.

1. Introduction

Foodborne diseases (FBDs) affect a large part of the population, becoming a problem to be faced, especially in underdeveloped countries (Mbonane & Naicker, 2020). Recent reports by the World Health Organization (WHO) estimate that around 600 million people fall ill due to FBDs per year, with a number of deaths that reaches 420 thousand worldwide, with children under 5 years being the most common group vulnerable, adding up to 125 thousand deaths per year (WHO, 2020). Among the main sources of infection and spread of FBDs are animal foods, such as meat and meat products (Gourama, 2020), since they are more susceptible to microbiological contamination that can come from the slaughter of the animal, where the carcass is excessively manipulated, or the influence of abiotic factors such as temperature, gaseous atmosphere and NaCl levels that select bacteria and allow their colonization on the meat surface (Rolfe & Daryaei, 2020; Stellato et al., 2016). Intrinsic factors such as nutrients, pH level, water activity and oxidation reduction potential also make meat a favorable environment to the colonization and development of a variety of microorganisms (Acuff & Ponder, 2020), mainly the deteriorating ones (Rodrigues et al., 2020), reinforcing the importance of monitoring in the production and commercialization stages of meat products (Song et al., 2019).

Marketing conditions can expose food to various microbiological hazards, where the hygiene of the place and the packaging temperature are important factors to be observed (Eshamah et al., 2020). In the commercialization stages, several sources of microbial contamination can be identified, including storage locations, utensils and the hands of the operators (Cardoso et al., 2021; Stellato et al., 2016). All of these factors bring about changes in the chemical composition and biological characteristics of the meat, which can result in unsafe food from a microbiological point of view (Pradhan et al., 2018).

The bacteriological quality of food can be established using microorganisms that indicate sanitary and hygienic conditions, such as the Coliform group, where *Escherichia coli* is the main representative (Bier et al., 2018; Fuka et al., 2021).

Also, it is common to investigate the presence of *Clostridium perfringens*, a gram-positive anaerobic bacillus, considered an important pathogen responsible for numerous diseases in humans and animals and which is constantly associated with food poisoning and systemic and enteric diseases worldwide (Hailegebreal, 2017). In addition to these, the search for indicators of contamination by organic material in food has proved to be of great importance, with *Pseudomonas aeruginosa* being the main agent identified as one of the most versatile and opportunistic, with its presence increasingly widespread in the environment (Mendonça et al., 2017; Silva et al., 2016).

Despite the countless evidences that demonstrate the importance of monitoring the bacteriological quality of meat and meat products in food safety (Stellato et al., 2016), studies that evaluate the effects of the retail type are little investigated as possible variables that affect the microbiological quality of these foods. Thus, in this study we analyzed meat samples collected under different conditions of trade, in order to identify the possible influence of management environments on the initial microbiological quality of meat products.

2. Methodology

The research was conducted in the municipality of Caruaru, Pernambuco, Brazil, and the samples collected in three butcher shops, three supermarket chains and in strategic points of the two main outdoor fairs in the municipality, from January to October 2016.

2.1 Sample collection and processing

48 samples of meat products sold at the COABH II and Parque 18 de Maio Fairs were collected, in three butcher shops and three supermarkets, with 19 samples of ground beef, 16 samples of pork sausage and 13 samples of chicken meat (breast, thigh and drumstick), according to the sampling plan described in Codex Alimentárius (Commission, 2007). These products were chosen based on the high frequency of consumption by the population of different social classes and because they are marketed in all environments analyzed.

The samples were transported in isothermal boxes at 4 °C to the Laboratory of Microbiological Quality Control of Food at Centro Universitário Tabosa de Almeida (ASCES – UNITA). The reduction of the crude sample occurred through the grinding of the food followed by its quartering, being discarded 2 / 4 of the total weight, thus generating the laboratory sample. Then, 100 g (50%) of each sample were liquefied in 300 mL of sterile saline using an industrial blender (METVISA LOL6) previously sterilized by autoclaving at 121 °C / 15min. All procedures were performed under sterile conditions in a laminar flow hood. From the broth obtained from the samples, aliquots were taken to carry out the bacteriological analyzes.

2.2 Bacteriological analysis

The analyzes followed the criteria stipulated by RDC n. 331 of 2019 from ANVISA (Brasil, 2019). The presence of total, thermotolerant coliforms, *P. aeruginosa* and *C. perfringens* was investigated, using the Multiple Tube Technique recommended by the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). For the identification and quantification of *C. perfringens*, the tubes were incubated in a jar with an anaerobic generator, providing the ideal atmosphere for bacterial growth, using Clostridium Differential Broth (CDB) for the detection of *Clostridium* sp. and Broth Litmus Milk for confirmation of the agent of interest.

2.3 Statistical analyses

The data obtained were expressed using the values found for the Most Probable Number of the microorganism of interest per gram of food (MPN/g), obtained through the Hoskins Table (APHA, 2012). The Kruskal-Wallis test was used to determine

significant differences ($p \leq 0.05$) between microbial contamination rates in meat samples sold at fairs, butcher shops and markets, using RStudio®, version 4.0.3, based on the type of methodological study described by Estrela (2018). To classify the degree of contamination, an index was created, developed in this study, stratifying the result into 5 levels, according to

Table 1, based on the MPN / g ranges, in order to establish the contamination index of the samples.

Table 1. Microbial contamination index expressed in MPN/g.

MNP/g	Contamination Index (CI)	Classification
<2	0	Negative
2 - 9	1.0	Low
11 - 90	1.1	Intermediate
110 - 900	1.4	High
1600 or >1600	1.7	Very high

Source: Authors (2017).

2.4 Ethical considerations

The research was carried out under aspects of extreme confidentiality, as well as the laboratory results analyzed. These were used for strictly academic, scientific and social service purposes, through continuing educational practices.

3. Results and Discussion

A high presence of microorganisms that indicate sanitary and hygienic conditions was observed in the samples of meat products in all analyzed marketing conditions (Table 2). Of the 48 samples, 83.33% revealed the presence of *C. perfringens* ($2x10^0 – 1.6x10^3$ MPN / g), with a higher prevalence in ground beef (89.5%), followed by pork sausages (81.25%) and chicken meat (76.9%). Fecal coliforms were present in 74% of ground beef samples, 62.5% of pork sausages and 84.61% of chicken meat. *P. aeruginosa* was present in 100% of all meat products analyzed. There was no significant difference ($p > 0.05$) in contamination between types of meat by the investigated microorganisms.

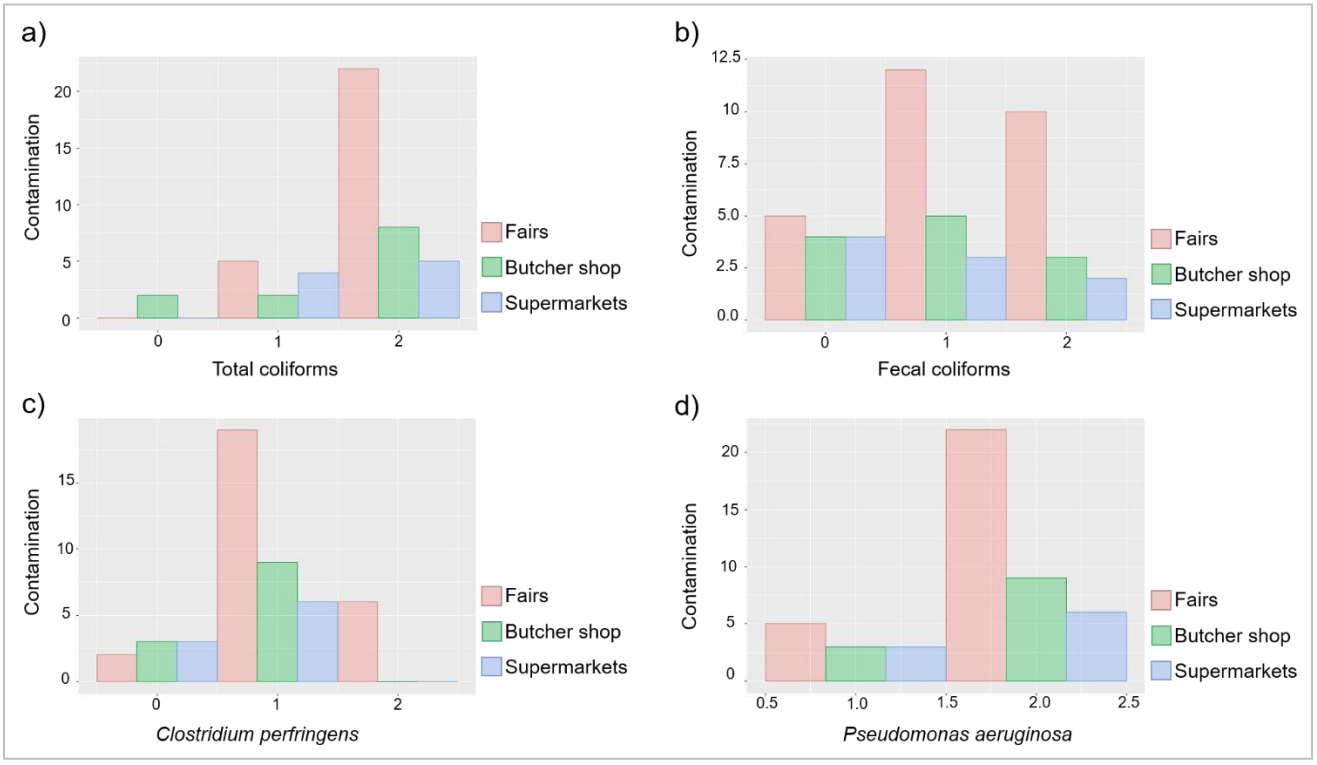
Table 2. Contamination index of meat products under different marketing conditions. Total (%): percentage of meat products samples contaminated and maximus and minimus values of MNP/g from the total of 48 samples collected (n = 48).

Trade	Microorganism	Meat	CI (%)	MNP/g	
				MIN	MAX
Fairs	Coliforms	Ground beef	83	2	1601
		Sausage	67	2	1601
		Chicken	100	2	1601
	C. perfringens	Ground beef	100	4	1601
		Sausage	78	6	600
		Chicken	100	2	1601
	P. aeruginosa	Ground beef	100	-	1601
		Sausage	100	9	1601
		Chicken	100	6	1601
Butcher shop	Coliforms	Ground beef	75	7	1601
		Sausage	50	2	1601
		Chicken	75	14	1601
	C. perfringens	Ground beef	75	11	350
		Sausage	100	26	350
		Chicken	50	14	170
	P. aeruginosa	Ground beef	100	240	1601
		Sausage	100	240	1601
		Chicken	100	50	1601
Supermarkets	Coliforms	Ground beef	33	-	1601
		Sausage	67	7	17
		Chicken	67	90	1601
	C. perfringens	Ground beef	67	-	2
		Sausage	33	4	11
		Chicken	67	2	7
	P. aeruginosa	Ground beef	100	900	1601
		Sausage	100	170	1601
		Chicken	100	-	1601

Score (-): not have minimum values. Source: Authors (2017).

As for marketing conditions, the Kruskal-Wallis test showed a statistically significant difference ($p \leq 0.05$) only in the contamination index by *C. perfringens* ($X^2_{(2)} = 8.5$; $p = 0.0143$). Dunn's *post hoc* test showed that meat sold at fairs (1.1 and 0.4, median and interquartile range) are more susceptible to contamination by the bacterial species than meat sold at supermarkets (1 and 0.9, median and interquartile range), ($p = 0.0125$). Although a higher rate of contamination of meat products by other microorganisms sold at fairs and butcher shop was observed in relation to supermarkets (Figure 1), there was no statistical difference between the marketing conditions (Table 3). There was also no significant difference in marketing conditions between the two fairs, so they were analyzed as a single group.

Figure 1. Contamination index (MPN / g) of meat products under different marketing conditions.



Source: Authors (2017).

Table 3. Analysis of variance of the bacterial contamination index under marketing conditions by the Kruskal Wallis test.

Microorganism	chi-squared	p-valor
Coliforms	1.2308	0.5404 ns
C. perfringens	8.4968	0.0143*
P. aeruginosa	0.5727	0.751 ns

ns – not significant; * statistically significant. Source: Authors (2017).

The marketing conditions in food of animal origin can intervene in the microbiological safety of them, being essential the monitoring of the sanitary and hygienic quality, in order to prevent outbreaks by FBDs (Pradhan et al., 2018; Stellato et al., 2016). Among the commercial places analyzed in this study, it was possible to observe that the meat sold in outdoor fairs had higher levels of contamination by the bacterial species investigated. In general, the sale of meat and meat products in outdoor fairs is a cause for concern and caution, since in these conditions food is exposed in environments that are often unhealthy and subject to contamination by microorganisms from the establishment and the surrounding environment (Magalhães et al., 2020). In the two fairs analyzed, meat products were sold at room temperature, poor hygiene conditions, exposed to vectors and with free access to consumers, who touched the meat during the purchase. Most traders did not use Personal Protective Equipment (PPE's) and all handled meat and money simultaneously during the sale. Similar practices were observed by Lima et al. (2017) when evaluating the hygienic and sanitary conditions in chicken cuts sold in Recôncavo da Bahia. They identified a correlation between the marketing conditions of informal retailers (fairs and butcher shops) with the presence of *Salmonella* spp., *E. coli* and total coliforms.

Municipal fairs are places with specific characteristics that have favorable conditions for the growth and proliferation of microorganisms in their environment (Silva et al., 2020). It is known that the main sources of contamination of food are: raw material (including water), environment (air, equipment, packaging and miscellaneous materials), and personnel (food handling) (Clark; Crandall; Reynolds, 2019). The problems found at the fairs are often related to the poor hygienic and sanitary conditions of the stalls (moldy, broken, damp, dirty, cracked), the traders (lack of PPE's and little training) and the products sold (history of contamination) (Magalhães et al., 2020). In view of the large number of existing fairs, adequate sanitary control is deficient due to the low number of employees to inspect all fairs. As they are not subject to effective inspections, it is not certain whether the product purchased has adequate quality and sanitary safety (Ghatak; Chatterjee, 2018).

Among the bacterial groups analyzed, *P. aeruginosa* was present in concentrations even higher than the results found in the research by Gu et al. (2016) who identified the presence of *aeruginosa* in 71.11% of meat samples, with an average count/g of 3.2×10^4. *P. aeruginosa* is known to play an important role in the deterioration of a variety of foods, including meat and meat products, since the aerobic storage of meat allows the growth of *Pseudomonas* spp. at different storage temperatures, presented greater competitiveness and consequently greater advantage in the growth rate over other bacteria under aerobic conditions (Gu et al., 2016).

The MPN/g values for fecal coliforms in this study were outside the limits established by Normative Instruction n. 60/19, which complements RDC n. 331 of 2019 from ANVISA (Brasil, 2019). When assessing the prevalence of microorganisms in chicken carcasses, supervised or not in the city of Viçosa, Brazil, Cossi et al. (2012) also identified high numbers of coliforms (2.99 log CFU / g) in the inspected samples and (2.54 log CFU / g) in non-inspected samples, suggesting that these results indicated poor hygienic and sanitary conditions. According to Adzitey et al. (2020) the contamination of meat samples by coliforms and *E. coli* indicates that lapses occurred during the slaughter, transport and commercialization of the meat, since when the animal is slaughtered, the muscles are exposed and can be contaminated by microorganisms such as *E. coli* from the gastrointestinal tract that rupture during evisceration.

As for contamination by *C. perfringens* in meat, Brazilian legislation (Brasil, 2019) determines only a limit for beef meat and chicken meat cooked or smoked. However, high rates of contamination by this microorganism in fresh meat can represent a major health hazard, since to achieve total spore destruction, longer thermal treatments will be necessary in order to prevent germination in food after cooking. Most of the outbreaks caused by *C. perfringens* are related to the consumption of cooked foods that have received insufficient heat treatment to destroy the heat resistant spores (El Kadri et al., 2020).

A high *C. perfringens* meat count was also observed by Rodríguez et al. (2002) who isolated the species in 75% of ground beef samples sold in retail meat markets in the Metropolitan Region of Costa Rica. The correlation between the contamination of meat by *C. perfringens* and the conditions of trade identified in our study corroborate the findings of Sabry et al. (2016) who, when evaluating the contamination of fresh and canned fish by *C. perfringens*, identified that the place of trade represents a risk factor for contamination of the external surface of fish from the surrounding environment.

The contamination of meat products by *C. perfringens* spores is more related to the moment of slaughter of the animal and to retail, where the place of sale, the temperature of meat storage, the equipment, cutting tools and the care by the handlers, are the main factors responsible for this contamination (Rodríguez et al., 2002). However, despite being considered a microorganism indicating remote fecal contamination, the presence of *C. perfringens* in large quantities has often been associated with serious episodes of foodborne infections worldwide (Hailegebreal, 2017; Sabry et al., 2016). Epidemiological evidence like this can be used both to promote measures to ensure food security for the population and to help notify companies responsible for food security offenses in such circumstances.

4. Conclusion

The three conditions of trade for beef, poultry and pork sausages showed contamination of these foods by the coliform group, by *C. perfringens* and *P. aeruginosa*. Lower levels of contamination were observed in products sold in supermarket chains, where the retail condition may have influenced the final quality of the product. Food sold at the two municipal fairs showed higher levels of contamination, as it is associated with the hygienic and sanitary conditions of these places. The high contamination of meat products by the bacteria investigated in this study demonstrates the great health risk involved in the consumption of these foods. These results can serve as a basis for health authorities in order to establish greater control over the microbiological quality of these commercialized foods, especially at outdoor fairs. More studies should be carried out with a larger sample number, in order to investigate the current conditions of these and new places of trade.

Acknowledgments

The authors would like to thank Centro Universitário Tabosa de Almeida (ASCES – UNITA), for the funding granted through the Scientific Initiation program, without which this work would not be possible.

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